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Effects of Hempseed Seed Oil Injection to Rats on Blood Lipoproteins Level

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ABSTRACT

Hempseed is a plant food source and an oily seed containing 25-35 % oil. Hempseed oil is rich with all types of plant sterols, which promotes health without any negative side effect. The current research will investigate the effects of intraperitoneal injection of the hempseed oil on male rats' blood serum profile and comparing it with its edible use.

Material and methods: To do so, 12 male rats were divided into four equal groups. In each group there were three replicate and three levels of 1.2, 1.3, and 1.4ml have considered. Injection proceeded within eight successive days and at the end, cupping took place through rats' neck vessel. Data obtained from testing biochemical factors in blood samples analyzed by SAS 9.3 software.

Results: Experimental results showed that the used hempseed had little pollution to THC and that injection of the oil affects faster than its edible use. The results show that 8-days injection of hempseed oil decreased factors such as total cholesterol (P=0.1737), triglyceride (P=0.1075), high density lipoprptein (HDL) (P=0.0049), uric acid (P=0.0001), alkaline phosphatase (P=0.0166), and albumin (P=0.0245) and increased low density lipoprotein (LDL) (P=0.0044).

Conclusions: This study showed that the sterol of hempseed oil had positive effects on decreasing of cholesterol, triglyceride, alkaline phosphatase enzyme, and uric acid while had no effects on other factors which is due to its pollution with a disturbing blood serum profile compound called delta-9-tertrahydrocannabino (THC). It can be found that hempseed had little THC contamination and injection affects faster than its edible use.

KEYWORDS: Alkaline phosphatase, Albumin, Hemp seed oil, Lipid profile, Phytoesterols, Uric acid.

INTRODUCTION

Scientists found out that lipoproteins and Apo lipoprotein levels are associated with risk of coronary heart disease (CHD). Epidemiological data demonstrates that increasing risk factor for CHD is relevant to increase in Low-Density Lipoprotein (LDL) cholesterol or apolipoprotein B (apoB) levels and decreases in HDL cholesterol or ApoA-I levels.[1-3]

The most important risk factor in the spread of cardiovascular diseases is abnormal amount of blood lipid. Random controlled studies suggest that decreasing lipid, lessens the risk of these diseases and death.[4] Risk of atherosclerosis and CHD is increased by the rise of blood serum total cholesterol and LDL-C.[5] Plant sterols or phytosterols exist in all plant foodstuffs and form a great amount of unsoponifiable fraction. High amounts of plant sterols prevent cholesterol absorption in human hence cause a modest decrease in serum cholesterol concentration.[6]

Reportedly, more than 200 different types of phytosterol exist in plants; the most opulent being β-Sitosterol, Campesterol and Stigmasterol. The main plant sterol natural resources for human diet is oil and margarine; although they also exist in vast ranges of seeds, grains, vegetables and unrefined vegetable oils.[7]

Despite the common believes, it is not a new method to use phytosterol to decrease serum cholesterol level. For instance, Peterson used Plant sterols in 1951 to feed chickens and decreased their cholesterol level.[8] In addition to the decreasing effects, anti-cancer characteristics, anti-atherosclerosis, anti-inflammatory and anti- oxidative effects were also shown in animals.[7]

Hempseed (Cannabis Sativa L.) is a grassy one-year-old

plant, which has been cultivated for years for its fiber and oil. It contains 20-25 % protein, 20-30 % carbohydrate, 25-35 % oil and 10-15 % fiber with a series of minerals.[9] It also contains vitamins (especially the tocopherols and tocotrienols of vitamin E series), phytosterols and amounts of B, C and D vitamins.[10]

Hempseed oil contains high percentage of unsaturated fatty acids such as 50-70% (w/w) linoleic acid, 15-25% (w/w) α-linolenic acid, 10-16% (w/w) oleic acid, 2-3% (w/w) stearic acid, 6-9% (w/w) palmitic acid and 1-6% (w/w) and linolenic acid.[11,12]

Hempseed oil is recommended for its 3:1 ratio of two unsaturated essential fatty acids (linoleic and linolenic acids). Natural products such as Beta sitosterol and Methyl salicylate, complete the nutritional characteristics of hempseed oil and increase its effects as a functional food.[11]

The use of plant foodstuff to improve serum lipid profile has recently been under many investigations. Myung et al. (1982) examined garlic effects on lipid metabolism in rats. According to their study using garlic for 4 weeks, resulted in a total cholesterol and Triglyceride levels decrease and high-density lipoprotein (HDL) levels increase in animal model.[13]

Edwards et al (1999) investigated effects of pistachio on Serum Lipid Levels in patients with Moderate Hypercholesterolemia. Total cholesterol, LDL-C and Triglyceride decreased and HDL-C increased after 3 weeks, Thus they concluded that use of pistachio improves serum lipid profile and the risk of cardiovascular diseases could be diminished (CVD).[14]

Sabate et al (1993) studied the effects of walnut on serum lipeid levels and blood pressure and discovered that using walnut for four weeks lowered LDL-C, HDL-C and Total cholesterol level. However, the LDL/HDL ratio, which has a positive correlation with CHD, decreased.[15] Many studies were done on other plants such as rice bran oil[16], fish oil[17], coconut and sesame oil[18] and black seeds (nigella sativa L.)[19] All showed remarkable effects on lipid profile in animal models.

Scientists have realized that unsaturated fatty acids decrease cholesterol level while saturated fatty acids have the inverse effect.[11,20-22]

In addition, many research led to the conclusion that plant phytosterols causes reduce in cholesterol absorption in competition with cholesterol to combine with biliary salts. Therefore, foodstuff rich with phytosterols can decrease serum cholesterol level.[23-26]

This study aims to extract oil, identify sterol compounds and inject it to male rats in order to examine its effect on decreasing level of total cholesterol, triglyceride, LDL-C, uric acid, alkaline phosphotase enzyme and increasing level of HDL-C and albumin which exist in rats' blood plasma, considering phytosterol characteristics and the full compounds of hempseed oil especially beta-sitosterol.

MATERIALS AND METHODS

The hempseed was randomly picked up from local market of Tehran. In this method seeds were cleaned and dried using oven (LDO-150T, LAB TECH Co, England) for 24 hours at 100℃. Cold pressed hempseed oil was obtained using a 10-kilogram capacity spiral press system (model 2008, NATIONAL ENG, South Korea).

Extraction of Unsaponifiables

1 ml of internal standard solution (α-cholestane solution of 1 mg/mL of acetone) was added to 250 mg of sample hempseed oil. 5 ml of ethanol potassium hydroxide solution (with concentration of 0.5 mol/L) (Merck, Germany) and a small amount of boiling stone was added afterwards. The reflux condenser (Model 2004, TCK Co., China) was attached to a 250 ml flask and the flask was heated for 15 minutes in order to boil the contents slowly.

Then heating was stopped, the flask's contents were immediately diluted with 5ml ethanol (Merck, Germany) and then shaked. The contents were then uniformed. 5ml of the solution was transferred to a prepared aluminum oxide column (Merck, German). The column was prepared by 10 g of aluminum oxide was suspended in 20 ml of solution , mixture was transferred to a 100 ml glass column (25cm H× 1.5cm ID) and aluminum oxide was let to be settled until the solvent surface reaches the upper layer of aluminum oxide and goes out of column.

This solvent was collected in a 50 ml round bottom flask. The unsaponifiables were first washed with 5ml ethanol and then 30 ml diethyl ether (Merck, German) was added 2ml/min. The solvent was then separated from flask by a reflux evaporator (Model 2006, EXERGIA Co, South Korea).

Thin layer chromatography (TLC)

The foresaid extracted unsaponifiables were solved in a little diethyl ether (Merck, Germany) and a line was drawn with that solution using 100 µL micro syringe 3 cm from the lower edge of TLC plate (silica gel (Merck, Germany)) with the dimension of 20×20 cm^2 and a 0.25 mm thick silica gel layer. Distance of 3 cm from edge of the plate was considered empty. 5µL of TLC standard solution (1mg/ml cholesterol in acetone and 5mg/ml betolyn in acetone (Merck, German)) was spotted 1.5 cm from the edge of the plate. The development tank (20×20 cm^2) was filled with about 100 ml of solution (to the equal volume fraction of hexane and diethyl ether), the TLC plate was placed in the tank and kept until solution reached to the upper edge of plate. The plate was then brought out of the tank and the solution in the surface of the plate was let to be evaporated under a hood.

Sterols separation

Methanol was sprayed on the plates in order to reach a white appearance of sterol in a darker background. Areas in the standard height spots were marked 2 mm higher and 4 mm lower than the visible points and this part of

silica layer was collected and transferred to a beaker by a palette knife.

Then 0.5 ml ethanol (Merck, German) was added to the collected silica gel. The silica gel in the beaker was mixed 3 times with the 5ml sections of diethyl ether and filled in a flask. The amount of ether in the solution was decreased to about 1ml using a rotational evaporator (R 308B, Senco technology Co, China) and the remaining solution transferred to a reaction container. The solvent was brought out with nitrogen gas flow (Tehran Gas Co., Tehran, Iran) through the 0.3ml reaction container.

Preparation of the trimethylsilylether Sterol

100µL silylation (50µL of 1-methyl imidazole (Sigma, German) was added to 1mL N-methyl-N-trimethylsilyl – hepta - floro butiramid) (Sigma, German) was added to a 0.3 ml reaction container which included isolated sterols. It was heated for 15 minutes in oven (LDO-150T, LAB TECH Co., England) at 105℃ in the closed container. Solution was then directly injected to a gas chromatograph (DB-5, HP Co, USA) after reaching to the room temperature.

Gas chromatography (GC)

Gas chromatography column (30×0.25mm ID×0.25µm), with hydrogen as gas carrying, flow rate of 36cm/s, two-part injection system with a ratio of 1 to 20, injection temperature and detector 300 and 320 respectively, temperature program was 240-255 and 4℃ per minutes speed and1µ injection volume were considered.

ANIMALS AND EXPERIMENTS

12 male rats weighing 210-250 g, were taken from faculty of pharmacy, Ferdowsi university of Mashhad, Mashhad, Iran. Animals were housed in individual cages in a 12 h/12 h light/dark cycle under standard laboratory conditions: temperature (25±1°C), humidity (55±5 %).They had free access to feed and clean water and stabilized for one week before the start of experiment. The study was conducted in agreement with the Guides for Care and Use of Laboratory Animals of the National Institutes of Health and all efforts were made to minimize animal suffering and the number of animals used.

STUDY DESIGN

All the rats were fed with chow diet during one week of adaption. And then they were divided into 4groups. Group A, B, C, D with hempseed oil of none, 0.1ml, 0.2ml, 0.4ml per day respectively. The process of injection was done using a 1-milliliter insulin syringe (Ava Pezeshk, Tehran, Iran).

The injections lasted for eight consecutive days with inter- peritoneal method and all groups were anesthetized with chloroform afterwards and then were killed and the blood samples were collected through neck vessel using pipes containing blood clot gel (Ava Pezeshk, Tehran, Iran).

BIOCHEMICAL ANALYSIS

Serum was obtained by high speed centrifugation (GYRO406G, GYROZEN Co, South Korea) for 15 minutes, 3000 rpm, then stored under -20C.

Measuring Total cholesterol

Cholesterol was determined through photometric method by standard kit (BioSystem, Spain).

The used reagents were sodium cholate 0.5mmol/L (Acros, German), phenol 28mmol/L, cholesterol esterase >0.2U/mL, cholesterol oxidase >0.1 U/mL, peroxidase >0.8 U/mL, 4-aminoantipyrine 0.5 mmol/L (Merk, German) and cholesterol standard was equivalent to 200mg/dL (Aldrich, German).

Sample and reagent were mixed thoroughly according to table1 and were incubated for 5 minutes at 37℃.The absorbance (A) of the standard and sample was measured by spectrophotometer (SQ4802, UNICO Co, USA) at 500 nm against the blank.

Table 1: Measurement of Total cholesterol

	Blank	standard	Sample
Cholesterol standard(S)	-	10µL	-
Sample	-	-	**10µL**
Reagent(A)	1.0 mL	1.0mL	**1.0mL**

Cholesterol was calculated by following general formula:

$$\frac{A_{sample}}{A_{standard}} \times C_{standard} = C_{sample} \qquad (1)$$

Measuring Triglycerides

The procedure and calculations were similar to measuring total cholesterol thus it was refrained from mentioning it again although the standard and reagent were different.

The used reagents were, 5 mmol/L Magnesium chloride, 6 mmol/L 4-chlorophenol (Aldrich, USA), lipase (Azmiran, Iran)>100U/ml, glycerol kinase (Merck, Germany) >1.5U/ml, glycerol 3-phosphate oxidase (Azmiran, Iran) > 4 U/ml, peroxidase>0.8U/ml, 0.75mmol/L 4-aminoantipyrine, 0.8 mmol/L ATP (Acrose, Germanyy), PH=7 and Triglyceride standard comprised of glycerol (Azmiran, Iran) equivalent to 200 mg/dl triolein (Merck, Germanyy).

Measuring Albumin

Serum Albumin was measured using photometric method by standard kit (ParsAzmon, Tehran, Iran).

According to table2; 10 µl of sample and 1000 µl of reagent were transferred into test tubes and were mixed thoroughly and incubated for 10 minutes at 37°C and within 60minutes, standard and sample's absorbance were measured against the blank .

Citrate buffer (PH 4.2) 30mmol/L (Merk, Germany), Bromocresol green 0.26mmol/L (Sigma, Germany) was used as reagents.

The following terms were considered for spectrophotometer (SQ4802, UNICO Co., USA):
Wave length: 546nm, temperature: 37℃, diameter: 1cm

Table 2: Measurement of Serum Albumin

	Blank	Sample or standard
Sample or standard	-	10 µL
Distilled water	10 µL	-
reagent	1000 µL	1000 µL

Albumin was calculated using the following formula:

$$\text{Albumin (g/dl)} = \frac{\text{Abs sample}}{(\text{Abs std/cal})} \times \frac{\text{conc.std}}{\text{cal(g/dl)}} \quad (2)$$

Unit conversion factor:
Albumin (g/dl) × 144.9 = Albumin (µmol/l)

Measuring HDL-C

To measure this factor photometric method was used with a spectrophotometer (SQ4802, UNICO Co, USA) and the standard kit (Pishtaz teb Co, Tehran, Iran).
The following reagents were used: Reagent1 (45ml) consist of N, N-bis-(4-sulfobutyl)-m-toluidine disodium 0.5mmol/L, cholesterol oxidase 1.0 U/ml, peroxidase, Good's buffer (PH 6.0) 50mmol/L (Merk, Germany) and Reagent2 (15mL) comprise of 4-aminoantipyrine 1.0 mmol/L, cholesterol esterase, detergent, Good's buffer (PH 6.0) 50 mmol/L (Merk, Germany).
The following terms were considered by Wave length 600nm, temperature 37℃ and diameter 1cm.

Table 3: Measurement of HDL-C

	Blank	Sample or calibrator
Reagent1 sample	900 µL	900 µL 10 µL
Reagent2	300 µL	300 µL

Reagent 1 and the sample were mixed (according to table3) and incubated for 5 minutes at 37℃, A_1 was read afterwards.
Reagent 2 was mixed with sample and incubated for 5 minutes at 37℃ then A_2 was read.
Concentration of HDL-C was measured by the following formula:
ΔA=A2-A1
(3)

$$\text{HDL (mg/dl)} = \frac{\Delta A \text{ sample}}{\Delta A \text{calibrator}} \times \text{calibrator concentration} \quad (4)$$

Measuring LDL-C

The kit and the method was similar to HDL-C but different in reagents. Reagent1 (45ml) contained 4-aminoantipyrine 0.5mmol/L, cholesterol oxidase 1.2 U/mL, cholesterol esterase, peroxidase, detergent, Good's buffer (pH 6.3) 50 mmol/L (Merk, Germany).
Reagent2 (15mL) consisted of N,N-bis-(4-sulfobutyl)-m-toluidine disodium salt 1.0 mmol/L (Aldrich,German), detergent, Good's buffer (pH 6.3) 50 mmol/L (Merk, Germany)

Measuring Alkalin phosphotase

To measure this factor DGKC (German society biochemistry) and standard kit (Pars azmoon Co, Tehran, Iran) were used.
Reagent1: diethanolamine (pH=9.8), 0.1 mmol/L, magnesium chloride0.5 mmol/L (Merk,Germany) and reagent2: p-nitrophenylphosphate 10mmol/L (Aldrich, Germany) were used and the considered terms for spectrophotometer were wave length, temperature and diameter which were 405nm, 37 ℃ and 1cm Single solution respectively.
After mixing as table 4, the amount of absorbed difference was read after 1minutes, the chronometer was enabled and differences in absorption from the previous minute was determined precisely after 1, 2, and 3 minutes.

Table 4: Measurment of Alkalin phosphotase

Sample : 20 µL
The mixed solution of 1 and 2 : 100 µL

The average was calculated and multiplied by the wavelength.
For the wavelength of 405nm: factor = 2757

Measuring Uric acid

To measure this factor photometric method with a spectrophotometer (SQ4802, UNICO Co, USA) and the standard kit (biosystem Co, Spain) were used.
Reagents used in this experiment were: phosphate 100mmol/L, detergent 1.5 g/L, dichlorophenolsulfonate 4mmol/L, uricase >0.12 U/mL, ascorbate oxidase >5 U/mL, peroxidase >1 U/mL, 4-aminoantipyrine 0.5mmol/L, PH 7.8 (Merk, Germany) and the uric acid standard equivalent to1 mg/dL (Merck, Germany).
The reagent was reached to room temperature, poured in a test tube containing the sample and was thoroughly mixed according to amounts in table 5; Then incubated for 10 minutes at 37℃ and the absorption rate for the standard and sample against the blank was read in 520nm.

Table 5: Measurment of Uric acid

	Blank	Standard	Sample
Distilled water	25 µL	-	-
Uric acid standard	-	25 µL	-
Sample	-	-	**25 µL**
Reagent	1 µL	1 µL	**1 µL**

The uric acid concentration in the sample is calculated using the formula (1).

STATISTICAL ANALYSIS

The one-way analysis of variance (ANOVA) and Toki test using SAS 9.2 statistical software was used to analyze the data. All values were expressed as mean ±SEM. Statistical significance was set at the minimum $p < 0.05$.

Table 6: Rats biochemical factors analysis results

Treatment	Oil	Uric acid	Cholesterol	Triglycerides	HDL	LDL	ALP	Albumin
Control	0	6.533^{a}	96	168	45.33^{a}	9.00^{bc}	617.33^{a}	4.13^{a}
Level	0.1	4.000^{c}	87.33	112	37^{b}	10.00^{ab}	455^{b}	3.73^{b}
	0.2	4.433^{c}	77	131.33	41^{b}	8^{b}	476.33^{ab}	3.76^{b}
	0.4	5.633^{b}	79.66	114	38^{b}	11^{a}	343.33^{b}	3.83^{b}
p-value		<0.0001	0.1737	0.1075	0.0049	0.0044	0.0166	0.0245
SEM		0.1414	5.8428	15.46	1.2018	0.4082	44.7139	0.0781
Normal statndards [26]		3.5-7.2	<200	<150	>60	<100	80-306	3.5-5.2

Note: $^{a, b, c, d}$ means with no common superscript within each column are significantly different. P<0.05: there is significant different between means in each column.SEM: Standard Error of the Mean. Uric acid, HDL, LDL, cholesterol and triglyceride: (mg/dl), ALP: (U/L), Albumin: (gr/dl).

RESULTS

Average moisture content of hemp seed and the yield of extracted oil was determined 7.9% and 25%, respectively. The GC chromatogram of hempseed oil is shown in Fig 1. More than 5 types of sterols were identified in hemp seed oil that is given in table 7. GC analysis of hempseed oil revealed that it contains about 6850 mg/kg of all types of esterols that the most abundant were about 67.65 mg/kg beta-Sitosterol followed by 14.91 mg/kg Campesterol. At the end 5mg/kg sterol has been extracted.

Table 7: Sterol composition of hempseed oil

Results	Property
	% Sterols
0.31	**Cholesterol**
14.91	**Campsterol**
4.40	**Stigmasterol**
67.65	**Total beta sitosterols**
12.73	**Other sterols**
6850	**Total sterol (mg/kg)**

Table 6, shows the result of biochemical factors in rats in groups A, B , C, and D after the injection of hemp seed oil. The weight of rats treated with hempseed after 8 day had no significant difference comparing to the first day.

The amount of total cholesterol (p=0.1737) and triglyceride (p=0.1075) in groups B, C and D decreased not significantly compared to A. However, the level of HDL in groups B, C and D lowered considerably (p=0.0049) comparing to A. This factor had no remarkable statistical difference in groups B, C and D.

8 -days hemp seed oil injection significantly increased LDL-C (p=0.0044) level in groups B, C and D compare to A. groups C and D had significant statistical difference so that amount of LDL-C in D, was more than all other groups. While C had least even to A**.**

Albumin level had remarkably decreased (p=0.0245) in groups B, C and D compared to A while statistically they had no notable difference.

Alkaline phosphatase enzyme level in groups B, C and D decreased noticeably (p=0.0166) compared to A. In group C it was more than B and D while in B and D the amount had no significant differences

Uric acid in groups B, C and D decreased significantly (p=0.0001) compared to A. The amount of this factor had no statistical difference in B and C while in D it showed outstanding rise.

Table 8: Comparing lipid factors in this research and similar cases

Reference	Total cholesterol	triglyceride	LDL-C	HDL-C
Isaac Karimi[27]	Increase	Decrease	Decrease	Increase
Hossein Hayatghaibi[28]	Increase	No change	Increase	No change
This study	Decrease	Decrease	Increase	Decrease

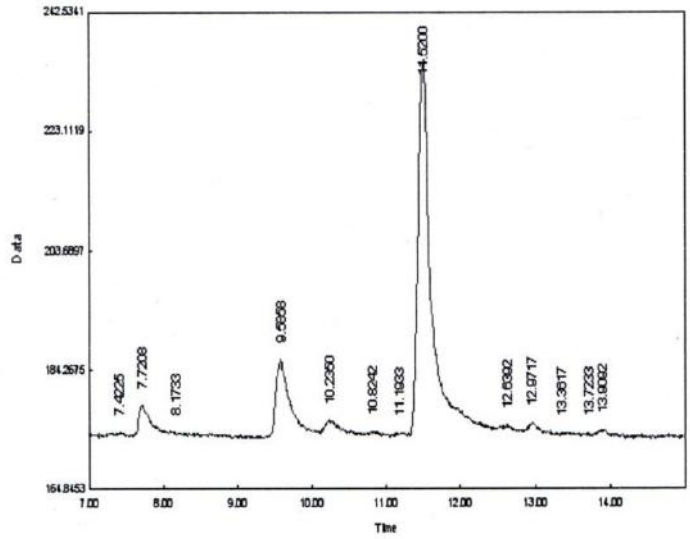

Figure1: Hempseed oil sterols chromatogram

DISCUSSION

Results obtained in this study and similar findings about serum factors can be seen in table 3. As mentioned in the table in this study, the total cholesterol level in groups B, C and D was decreased not significantly compared to group A; which is in contrast with Karimi (27) and Hayatghaibi (28) studies within which the total cholesterol level was increased. Also the Triglyceride level which was decreased not remarkably in B, C, and D groups in comparison with group A, contradicts Karimi and Hayatghaibi researches' results, which showed increase without any changes.

In addition, the HDL-C level in groups_B, C and D decreased noticeably compared to A, which again is in contrast with findings of Karimi and Hayatghaibi that showed increase in this factor.

The LDL-C level in B, C and D showed significant increase comparing to that of group A which is in contrast with Karimi that showed a decrease in LDL-C, but it was confirming Hayatghaibi's results.

Another factor under-study in this research is Albumin that decreased significantly in groups B, C and D compared to A. It is again in contrast with Karimi research, in which Albumin level increased not significantly. Albumin is one of the major products of protein synthesis in liver. Many studies have been done on relation between amount of serum albumin and CHD and scientists have found a negative correlation. So that the more decrease serum Albumin level has the higher risk of CHD.[29-31]

Also in this study, alkaline phosphatase enzyme level in B, C and D was decreased remarkably compared to A. Alkaline phosphatase is an enzyme which is mainly produced in liver and marrow. Its rise in serum causes severe damage to heart and lung tissue, kidney and inflammation of digestive system .while its reduction leads to dangerous anemia and lack of C, D and B_6 vitamins.[32,33]

In this study serum uric acid in rats was also examined and the result was its meaningful decrease in B, C and D groups compared to A. Uric acid is produced because of purine metabolism and it often exists in high amount in cardiovascular and coronary patients.[34,35]

Also increase of blood pressure rises serum uric acid which may be the kidney's blood circulation decrease result.[36,37] In addition, rise of uric acid may cause early kidney problems in patients with type2 diabetes, which may be the result of acid deposit on the tissues.[38]

Results obtained through this study showed that 8 -days hempseed oil injection has positive effects on factors such as total cholesterol, triglyceride, alkaline phosphatase enzyme and uric acid but no effect on HDL-C, LDL-C and albumin. Comparing this study with that of Karimi and Hayatghaibi, intra-peritoneal injection of hempseed oil has better and faster effects on these factors.

Concerning the levels for injection in B, C and D groups, it seems that at the highest level of injection, factors such as uric acid, total cholesterol, LDL-C and Albumin increased and Alkaline phosphatase decreased remarkably in comparison with B and C .This may express that in order for to this oil to be effective on improving blood serum profile and having useful results for this oil, special levels should be considered. Also, according to Karimi and hayatghaibi, uselessness of hempseed oil injection on some factors can be attributed to a "delta-9tertahydrocannabino (THC) compound.

This compound is an antioxidant which is appetizing. Lipid disorders are caused by THC contaminated hempseed. Based on Szmitko[39] studies, feeding rat with the hempseed, leads to serum lipid increase. The more the hempseed being free of this compound, the better

effect it puts on serum profile. Type of plant and degree of its pollution while harvesting, are important factors to determine THC contamination within the seed.[40]

Considering positive effects of the examined seed except for three factors, it can be said that the oil-extracted contains a few of this compound.

CONCLUSION

This study shows that hempseed oil injection had positive effect on some of serum factors so that meaningless decrease of cholesterol, triglyceride and meaningful decrease of uric acid and alkaline phosphatase occurred. Also, this injection led to meaningful increase in LDL and meaningful decrease in HDL and albumin. The cause of hempseed oil being ineffective on some factors can be attributed to THC compound, which disturbs serum profile. The more hempseed is polluted with THC, the less it affects examined factors. Also, we found out that intra-peritoneal injection of the oil affects faster rather than its being eaten.

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